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Distribution/Spread of Superbug and Potential ESKAPE-B Pathogens amongst Domestic and Environmental Activities: A Public Health Concern

Igere BE[1,2,✉], Peter WO[1], Beshiru A[1]

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Author Affiliation:
[1]Department of Microbiology and Biotechnology, Western Delta University Oghara, Delta State, Nigeria
[2]Applied and Environmental Microbiology Research Group, Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5700, Eastern Cape, South Africa

✉**Corresponding author:**
Email: igerebright@wdu.ed.ng; 201710685@ufh.ac.za; ibe22002@yahoo.com;
Tel.: +27-733491868; +2348038792425.

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ABSTRACT

Potential bacterial pathogens and resistant strains continue to spread and have been implicated in diverse infections of man, animal and plants. Most of these strains have been source-track to inhabit environmental nexus and their spread has become an environmental and public health concern. This study examined the distribution/spread of superbugs and potential ESKAPE-B pathogens among domestic activities. The study was conducted particularly on two assessment clusters; determination of human subject awareness on the spread of superbugs and the laboratory isolation, characterization, antibiogram and prevalence of the various organisms in selected domestic activities sources. Data were collected using structured questionnaires while swab samples were collected from respondents irrespective of their previous activities. Swab samples were collected from vehicles seats and doors handles, classrooms chairs, offices chairs, tricycles, bicycles, toilets WC as well as gymnastic centre within the four local communities of Oghara. Swab specimens were cultured onto sterile MacConkey agar, Centrimide agar, Salmonella-Shigella agar and Mannitol salt agar on every sampling and incubated at 37^0C for 18-36 hours. Organisms observed were purified, stored on agar slants, Gram-stained and characterized using biochemical test simultaneously. The study reveals that majority of respondents in the study area do not apply appropriateness in hygienic practice which is a potential source to the spread of resistant bacteria. It was also evident that *P. aeruginosa* (21/132; 15.9%) is most prevalent in the environment including other members of the ESKAPE-B strains. Isolates show resistance phenotype as follows: Amoxicillin (100%), Ceftazidime (86.5%), Cefixime and Cefuroxime (98.1%), Ofloxacin and Ciprofloxacin (96.2%), Gentamicin (100%). Plasmid Analysis revealed that amongst 132 strains of ESKAPE-B as well as *Vibrio specie*, *Salmonella specie* and *Shigella specie* retrieved during the study, 62.9% (83/132) were shown to harbour plasmid of size ranging from 22kb to 1kb, indicating that some of the resistance observed from the antibiotic susceptibility testing were associated with resistance plasmids. Our observation has shown that the distribution and spread of these antibiotic resistant bacteria is associated with poor implementation of standard hygienic practice and poor awareness. The need for an adroit implementation and application of standard hygienic practice cannot be overemphasized.

Keywords: ESKAPE-B potential pathogens; Superbug; Antibiotic Resistance strains; Plasmid profiling; Domestic materials; Questionnaire

1. INTRODUCTION

Superbugs is a term used to describe bacteria strains that are resistance to major antibiotics commonly employed for therapeutic management/control of bacterial implicated infections today (CDC, 2017). Bacterial are shown to be implicated in diverse infections including systemic and superficial infections eg pneumonia, urinary tract infections, upper respiratory tract infections, acute watery diarrhea/cholera, wound infection (burn, gun, accident etc). Other skin infection and systemic infections are just a few of the dangers we now face. In recent times, such strains have elicited resistance to multiple antibiotics which earned the acronym MDRB (multidrug resistant bacteria). Some of them have been considered as extensive drug resistance (XDR) strains, totally drug-resistant (TDR) strains and/or Pan drug resistance (PDR) bacteria. According to the Infectious Diseases Society of America (IDSA), MDRB is a term used to describe bacterial that are non-susceptibility to at least one agent in three or more antimicrobial categories. "XDR is used to describe bacteria that are non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories)" and "PDR is used to describe bacterial that are non-susceptibility to all agents in all antimicrobial categories". To ensure correct application of these definitions, bacterial isolates should be tested against all or nearly all of the antimicrobial agents within the antimicrobial categories with selective reporting and suppression of results should be avoided (IDSA, 2012). Due to the aforementioned expressions by members of bacteria species, the IDSA and Centre for Disease Control (CDC, 2009) proposed a solution policy report, were organisms that exhibit the above resistance features are referred to as "Bad Bugs, or Superbugs" since there are no useful drugs/antibiotic against such organisms. The policy also emphasized a moribund tendency on research and development in novel antibiotic discovery, Public Health Crisis Brews", and spread of such resistance character persist (Holmes *et al*.,2016; IDSA, 2012; CDC, 2009). Recently, there was a "Call to Action" on how to provide an update on the scope of the problem of superbugs, spread and the possible solutions.

Currently, scientific research investigators have initiated interest on the concerns made by IDSA and CDC. One of such area of concern that arouses interest as indicated by the call to action was the spread of superbug. Bamberg and his colleagues in addition to other investigators have reported that the spread of resistant bacteria occurs both in the hospital and the environment (Bamberg *et al*., 2014; Rice, 2010). This implies that processes and activities in the hospital system (health care system) and the environment have been implicated as major sources/spread of drug resistant infections. It is also affirmed that a certain group of bacteria cause most of the antibiotic resistant infections in hospital settings and the environment. The Infectious Diseases Society of America (IDSA) continues to view with concern the lean pipeline for novel therapeutics to treat drug-resistant infections associated with superbugs, especially those caused by gram-negative pathogens. The IDSA recently highlighted a clique of such microorganisms as ESKAPE-B pathogens' – capable of 'escaping' the biocidal action of diverse antibiotics and collectively representing new paradigms in multiple antibiotic resistance. This group of bacterial as it is called the ESKAPE-B pathogens, will be of increasing relevance to antimicrobial chemotherapy in the coming years, not only due to the clinical threat they pose, but the anticipated honing of environmental, academic and industrial interests towards them. This acronym is given according to the definition to encompass the enterobacteriaceae, Gram positive and other Gram negative bacteria (IDSA, 2017). These bacterial includes: *Enterococcus faecium, Staphylococus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter specie* and *Bulkholderia cepacia*. Initially the acronym was "ESKAPE" but as time went on and resistant nature continues to be a burden both in environmental associated infection and infected hospitalized patients, *Bulkholderia cepacia* which today is assuming the superbug nature was then included as several reports as well as investigators have shown (William and Shiel, 2018). The reoccurring concerns reported on resistant bacteria in the environment with the advent of multiple antibiotic resistant bacteria and the zero awareness on the origin of such resistant strains (source track) especially amongst developing countries continues to arouse interest of researchers in such related field. As a strategy for awareness, sustainability of the environment, control and public health concerns of such fast spreading organisms in apparently docile underdeveloped environment, the study was conducted to determine the distribution/spread of superbug and potential ESKAPE-B pathogens amongst domestic and environmental activities: a public health concern. This is done to study their distribution, prevalence, origin and reduce the growth of these superbug in the environment. This study attempts to generate original local data and examine the magnitude of drug resistant organisms in the study environment. In addition, it also serves to call a prompt detection procedure which is necessary for implementation of stringent infection control policies. The study focuses specifically on some domestic activities that portend their growth, spread and

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how to reduce/control their proliferation. Some of these focused pathogens includes: *Enterococcus faecium, Staphylococus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter specie and Bulkholderia cepacia.*

2. MATERIALS AND METHODS

2.1. Study Area

This short term cross-sectional study was conducted in the department of Microbiology and Biotechnology, Western Delta University Oghara, delta State Nigeria from 15th of April to 15th of December, 2020. The study was conducted within the suburban area of the Western Delta region of the State and Western Delta University. Four communities of the region were selected for the study including Ajagbodudu, Oghara-efe, Oghara-eki and Otefe. These communities were selected based on a presumed interest and susceptibility to possible outbreak of relative superbug (ESKAPE-B) or multiple antibiotic resistant strains. It was also observed that populace in the study environment lacks awareness on interpersonal hygiene and standard hygienic practices. It's located at latitude (E 057°C and W 047°C) of the region.

2.2. Questionnaire design and quantitative data collection

A structured questionnaire was prepared based on reviewed relevant literatures in English language for data collection. The questionnaire is designed to examine respondent views on diverse activities that may aid the spread of superbug (multi drug resistance organisms) which causes more harmful diseases to human health. It's divided into two main section and six sub-sections with twenty-four questions (Appendix 1).

2.3. Ethical Consideration

An ethical clearance was approved on the 7th October 2019 by the Department of Microbiology and Biotechnology Research Ethics Board with reference number WDUMCB/2020/ECCvol/140.

2.4. Sample Collection and Isolation of Bacterial Strains

Swab samples were collected from respondents irrespective of their travel history from the following; seats and doors handles of vehicles, classrooms chairs, offices facilities, tricycles/bicycles seats, toilets WC as well as surfaces of gymnastic centre facilities within the four local communities of Oghara which has an average population of 157,417. The bacterial strains were isolated from different samples and were identified by standard and conventional Microbiological methods (Washington *et al*., 2006; Cowan and Steel, 1975).

2.5. Isolation of species

Swab specimens were inoculate onto MacConkey Agar, Kligler Iron agar, Mannitol Salt Agar, Centrimide Agar, TSB agar, Blood Agar and Eosin methylene blue agar (E.M.B) and incubated at 37 °C for a 24 hours, as described by Iweriebor *et al* . (2015), Cowan and Steel, (1975), Harley and Prescott, (2002). Black colonies, yellow colonies, pyocyannin pigmenting colonies, non-lactose fermenting colonies and pinkish colonies were considered as presumptive isolates and one colony per plate was used for sugar fermentation and biochemical characterisation.

2.6. Standard Typed Cultures Used

Some standard typed organisms were used during the study as control strain. There were treated in the same way as suspected clinical isolates from specimen in other to ascertain the identity and susceptibility of the isolates. These are *Escherichia coli* K-12 strain which was used as Plasmid DNA recipient strain during transformation (Transconjugation), while *Pseudomonas aeruginosa* ATCC 27853, *E. feacalis* ATCC 19433 and *Acinetobacter calcaoceticus* anitratus CSIR were used as control for identification and susceptibility testing. Observed growths of organisms were collected, purified, stored on prepared agar slants and Gram-stained simultaneously.

2.7. Analysis of Samples

The specimens were collected at an interval of three months. For each sampling, swab sticks were taken to the laboratory immediately for microbiological analysis. Swab sticks from various locations were cultured onto sterile blood agar, MacConkey agar and kligler iron agar (see appendix for composition) on every sampling and incubated aerobically at 37 ^{0}C for 18-36 hours. The morphological characteristics of the colonies such as shape, colony arrangement, capsules, spore, motility, bud or sheath, pigmentation and other cultural characteristics on agar plates (Nutrition, temperature, of growth, forms, margins, elevation and

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density) were taken into cognizance. Observed colonies were Gram stained and separated into Gram positive cocci and Gram negative bacilli (Cowan and Steel, 1975; Tille *et al*., 2014; Harley and Prescott, 2002).

2.8. Growth at Elevated Temperature (42 ^{0}C)

Suspected colonies were subcultured onto Nutrient agar and MacConkey agar, which were then incubated at 42 ^{0}C for 18-24hours. After the incubation, isolates were further tested with biochemical reagents for their activity (Cowan and Steel, 1975).

2.9. Motility Test

The hanging drop method was applied, by making a ring of say 18mm diameter with a plasticine on a clean grease free slide and emulsifying an inoculum from an overnight broth culture of the suspected organism at the centre of cover slip. With a gentle press, place the ring of the plasticine onto the cover slip to centralize the innoculum. Then with sharp movement, invert the slide so that the cover slip will be at the uppermost side and examine microscopically using X10 objective lens to focus and X40 objective lens to view (Cowan and Steel, 1975; Tille *et al*., 2014; Harley and Prescott, 2002).

2.10. Biochemical Reaction and Characterization of Isolates

A Battery of biochemical tests was applied. The isolates were purified and the procedures for use were followed according to manufacturers' specifications. The identity of isolates was determined using Bergey's manual of determinative bacteriology (volume 4, 2004).

2.10.1. Urease Activity

A sterile agar slope/slant of the urea medium (Christensen's urea medium) was inoculated with an overnight culture of the suspected organism and incubated overnight. Urease activity is indicated by the production of red colour at the surface of microbial growth (Cowan and Steel, 1975; Harley and Prescott, 2002).

2.10.2. Indole Test (Kovacs Reagent)

Suspected colonies were subcultured onto a nutrient broth and incubated overnight. To the overnight both culture of the isolates, few drops of Kovac's reagent were added and indole production was indicated (shown) by the production of red ring at the surface of the medium (Cowan and Steel, 1975; Harley and Prescott, 2002).

2.10.3. Citrate Test (Koser's Medium See Appendix)

Few drops of the peptone water suspension of the suspected isolate was asceptically inoculated onto Simmon citrate medium, and it is incubated overnight (18-24hrs) at 37 ^{0}C. citrate utilization was indicated by the change in colour into the medium from light green to Turkish blue (Cowan and Steel, 1975; Harley and Prescott, 2002).

2.10.4. Oxidase (Cytochrome C Oxidase) Test

The dry filter paper method was used since oxidase reagent is unstable. Colonies of the suspected organisms were smeared on the whatman No 1 filter paper soaked with oxidase reagent (1% teramethyl-p-phenylenediamine dihydrocloride) stored in ice. Oxidase production was indicated (shown) by a change of colour of the smear to deep purple within 5 seconds (Harley and Prescott, 2002).

2.10.5. Catalase Test

This was carried out according to the method recommended by Cowan and steel (1965). A drop of 3% hydrogen perioxide (H_2O_2) was placed on a clean grease free slide. Onto this, a colony of the test organism was emulsified with the aid of a glass rod. It was observed within few seconds and the production of bubbles indicating release of oxygen from the mixture is an indicator for positive reaction (Harley and Prescott, 2002).

2.11. Phenotypic Detection of virulence characteristic

2.11.1. Detection of haemolysin

Pure presumptive isolates were cultured onto blood agar. After about 18-24hours incubation, an observation of lysis of the red pigment around the region surrounding the growth of organism indicates positive haemolysis. This technique involves inoculating a loopful of an overnight test organism onto blood agar by streaking and incubated at 37 °C for 24 hr. Zone of haemolysis (β-haemolysis) appearing around colonies indicates a positive test (Elliot *et al.,* 2001; Miyagi *et al.,* 2016).

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2.11.2. Detection of protease

It was performed on skimmed milk agar (see section above) as described by Benson (2002). A single colony of a 24 hr culture from Nutrient agar was inoculated onto the surface of prepared skim milk agar and incubated at 37 °C for 24- 48 hr. The appearance of clear zone of hydrolysis around inoculum indicates positive test (Igere et al., 2020).

2.11.3. Detection of Lipase:

The method of Rhodina (1972) was employed using a constituted medium to detect lipase production. Media constituents are 5 gm H_2HPO_4, 5gm $(NH_4)_2PO_4$, 1 gm $CaCl_2.6H_2O$, 1 gm $MgSO_4.7H_2O$, 0.001 gm NaCl, 0.001 gm $FeCl_2.6H_2O$, 5 ml Tween 80, 20 gm Agar and 900 ml de-oinised water. After preparation, the pH is adjusted to 7.2 and volume adjusted to 1L, allowed to dissolve completely with gentle heat and magnetic stirrer and finally sterilized by autoclaving at 15 min at 15 pound per square inch at 121 °C. Sterile content were allowed to cool to 45°C, poured onto sterile petri dishes allowed to polymerise and maintained at temperature 4 C° until further used. As described, the surface of prepared media was inoculated with a single colony of an overnight test organism and incubated for (1-5) days at 37 °C. The appearance of turbid zone around colonies indicates a positive result.

2.11.4. Antibiotic Susceptibility Testing

Antimicrobial susceptibility test of the various isolates was performed using the standard disk diffusion test also commonly known as Kirby-Bauer test. Mueller-Hinton agar plate and paper disk containing a single concentration of antimicrobial agent was place centrally on the surface of the agar plate containing the isolate and incubated for 18 – 24 hours in an incubator (37^0C). Antibiotics used for Gram positive cocci (GPC) were Amoxicillin, Ofloxacin, ciprofloxacin, amikacin and levofloxacin while those for Gram negative bacilli (GNB) were amikacin, ceftazidime, cefuroxime, cefixime, Amoxi-clavulanic acid (Augmentin), gentamicin, Nitrofurantoin and ciprofloxacin respectively. Piperacillin-Tazobactam and Sulbactam-Ampicillin were used as double synergy test. For Quality Control of antibiotic susceptibility test, *Staphylococus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were used. MDR, XDR, and PDR strains were detected as per criteria described by CLSI and EUCAST (CLSI, 2015; Matuschek et al., 2014; Magiorakos et al., 2012). Extended Spectrum β-lactamases (ESBL) producing strains were detected by combined disk method using ceftazidime (30 μg) and ceftazidime plus clavulanic acid (30 μg plus 10 μg) (Igere *et al*., 2020; Carter *et al*., 2000). An increase in diameter of ≥5 mm with ceftazidime plus clavulanic acid as compared to ceftazidime disk alone was considered positive for ESBL detection. Results are interpreted using a standard method published by National Committee for Clinical Laboratory Standards (NCCLS) now known as Clinical Laboratory Standards Institute (CLSI, 2015; Matuschek *et al*., 2014; Magiorakos *et al*., 2012).

2.11.5. Determination of Multiple Antibiotic Resistance (MAR) index

Multiple antibiotic resistant index (MARI) of resistant isolates was determined according to the method of Odjadjare *et al*. (2012) and Igere et al., (2020) as described below:

MARI = A/B

where;
A = number of antibiotics to which the isolate was resistant
B = total number of antibiotics to which the isolate was exposed.

2.11.6. Plasmid Isolation

Plasmid DNA was extracted from bacterial cells, which were harvested from solid media and applying the basic steps such as cell harvest, cell lysis, cell deproteination, cell decontamination. Extracts were eletrophoresed using a Sigma-based tris acetate-EDTA (TBE) of 50× (Sigma Aldrich, Dorset, UK), 1X TBE running buffer and agarose powder. Gel was prepared by dissolving 0.8 g of agarose powder (Sigma Aldrich) in 100 mL of running buffer and heated to boiling. The prepared gel is casted on a minigel tray (Anachem, Dorset, UK), allowed to polymerise, placed carefully in an electrophoresis tank filled with 1 × TBE Buffer and electrophoresed (electrophoresis machine CLS-AG100, Warwickshire, UK) at 100 V for 50 min. The gel was visualized on a Gel doc imaging system (Bio Rad Hercules, Califonia, USA).

2.11.7. Statistical analysis

All experiments were performed in duplicates and the results expressed as percentage in tables and figures using Microsoft Excel.

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3. RESULTS

The result of our study was presented in figures/photos, Tables, histogram and heat-map as shown below. Table 1 shows the demographic reports of respondents, it revealed that the age with the highest respondents was 26 -30 years, with majority of them been students (70), and are single on marital status and mostly belong to the Urhobo. Table 2 shows the domestic activities, water source and disease pattern of the populace. Their major water source is others (52) which include river; well etc. the water is collected mainly by bottles (50), and stored in tanks (60), their water sources are not treated in the appropriate/standard methods with some of them still employing the random disposal of feces (13).

Table 1 Demographic results of respondents

Age	16-20 (30)	21-25 (10)	26-30 (36)	31-35 (8)	36-40 (16)	41-above (2)
Sex	Male (60)		Females (42)			
Occupation	Students (70)	Drivers (20)	Gymn Centre Instructors (2)	No Occupation (10)		
Marital Status	Married (30)	Single (72)				
Religion	Christianity (100)	Islam (2)				
Ethnicity	Igbo (21)	Hausa (11)	Benin (10)	Urhobo (60)		

The table 1 describes the demographic reports of respondents with the maximum age range of respondents as 26 – 30.

Table 2 Domestic activities, Water source and Disease pattern of the Populace

Domestic activities	Travelling (32)	Education (60)	Visitation (10)		
Domestic water sources	Tap water (40)	Well water (10)	Others (52)		
Water Treatment	Boiling (12)	Chlorination (10)	Filtration (20)	Others (60)	
Water collection	Bucket (20)	Cup (12)	Bottles (50)	Cans (10)	Others (10)
Water Storage	Cans (20)	Drum (15)	Tank (60)	Others (7)	
Water related disease	Yes (94)	No (8)			
Disease type	Cholera (20)	Diarrhea (32)	Vomiting and Stomach pain (40)	Others (10)	
Toilet facility	Local Pithole (40)	WC Standard (49)	Random Disposal (13)		

The table 2 describes the domestic activities of respondents with the maximum as travelling and domestic water sources as others

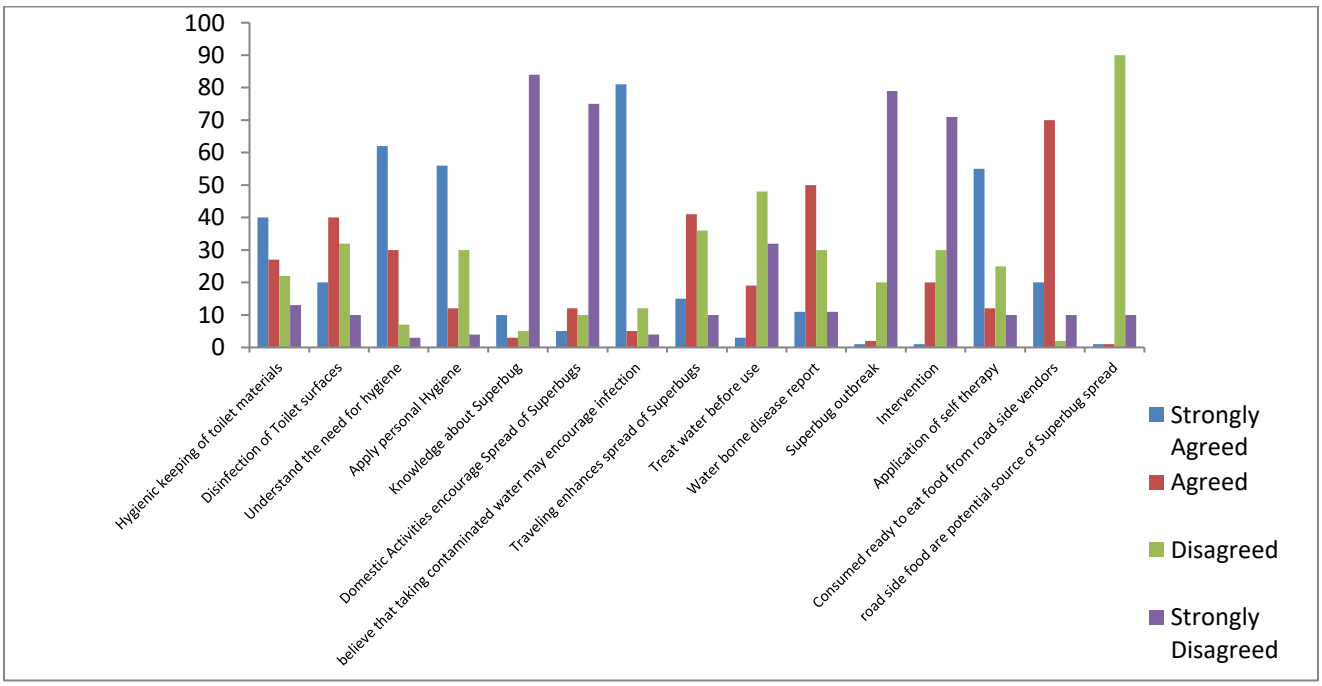

Figure 1 Shows the Safety practices of hygiene during Domestic Activities and Healthy Practices

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The figure 1 shows the safety practices of hygiene applied by respondents during their domestic activities and healthy practices. It revealed that majority of the respondents does not have any prior knowledge of superbugs or multiple antibiotic resistant organisms and domestic activities may encourage the spread of superbugs. It also reveals that consumption of road side ready to eat foods are potential agents of spread of multiple antibiotic resistant bacterial or superbugs. Other reports of the bacterial strains observed during the study, site and percentage occurrence of presumptive isolates retrieved during study, cultural morphology/characteristics, Gram reaction, antibiotic susceptibility testing report, and plasmid profiling reports are presented in Tables 3 to 4 and figures 1 – 10.

Table 3: Site and Percentage Occurrence of Presumptive Isolates Retrieved during Study

Site/Number of Specimen collected	Type of Presumtive organism retrieved	Numbers of Isolates retrieved	% isolate retrieved per site	Total % isolates Retrieved
Vehicle/car seats (22)	Enterococcus faecium	4	12.5	3.03
	Staphylococcus aureus	5	15.6	3.79
	Klebsiella pneumonia	3	9.4	2.27
	Acinetobacter baumanii	3	9.4	2.27
	Pseudomonas aeruginosa	5	15.6	3.79
	Enterobacter specie	3	9.4	2.27
	Bulkhoderia cepacia	4	12.5	3.03
	Vibrio specie	2	6.3	1.52
	Salmonella specie	2	6.3	1.52
	Shigella specie	1	3.1	0.76
Gym Centre (19)	Enterococcus faecium	4	13.8	3.03
	Staphylococcus aureus	4	13.8	3.03
	Klebsiella pneumonia	2	6.9	1.52
	Acinetobacter baumanii	1	3.5	0.76
	Pseudomonas aeruginosa	4	13.8	3.03
	Enterobacter specie	3	10.4	2.27
	Bulkhoderia cepacia	4	13.4	3.03
	Vibrio specie	3	10.4	2.27
	Salmonella specie	2	6.9	1.52
	Shigella specie	2	6.9	1.52
Classroom Seats (26)	Enterococcus faecium	4	18.2	3.03
	Staphylococcus aureus	4	18.2	3.03
	Klebsiella pneumonia	2	9.1	1.52
	Acinetobacter baumanii	2	9.1	1.52
	Pseudomonas aeruginosa	3	13.6	2.27
	Enterobacter specie	2	9.1	1.52
	Bulkhoderia cepacia	2	9.1	1.52
	Vibrio specie	2	9.1	1.52
	Salmonella specie	1	4.6	0.76
	Shigella specie	0	0	0
Toilet materials (23)	Enterococcus faecium	3	12.5	2.27
	Staphylococcus aureus	3	12.5	2.27
	Klebsiella pneumonia	1	4.2	0.76
	Acinetobacter baumanii	2	8.4	1.52
	Pseudomonas aeruginosa	4	16.7	3.03
	Enterobacter specie	0	0	0
	Bulkhoderia cepacia	2	8.4	1.52
	Vibrio specie	3	12.5	2.27
	Salmonella specie	4	16.7	3.03
	Shigella specie	2	8.4	1.52

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Door handles (24)	Enterococcus faecium	2	8	1.52
	Staphylococcus aureus	4	16	3.03
	Klebsiella pneumonia	2	8	1.52
	Acinetobacter baumanii	3	12	2.27
	Pseudomonas aeruginosa	5	20	3.79
	Enterobacter specie	3	12	2.27
	Bulkhoderia cepacia	2	8	1.52
	Vibrio specie	2	8	1.52
	Salmonella specie	1	4	0.76
	Shigella specie	1	4	0.76
TOTAL (114)		**132**		

Figure 2: Shows the swab sticks collected and used during the study

Figure 3: Gram negative bacilli observed using binocular microscopy during study

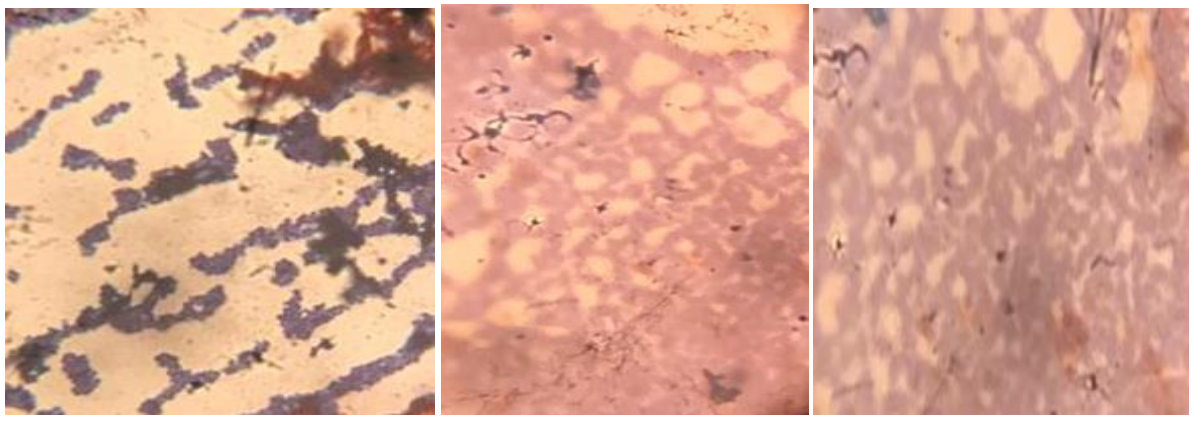

Figure 4: Gram Positive cocci observed using binocular microscopy during study (typical for *Enterococcus* and *Staphylococcus* species)

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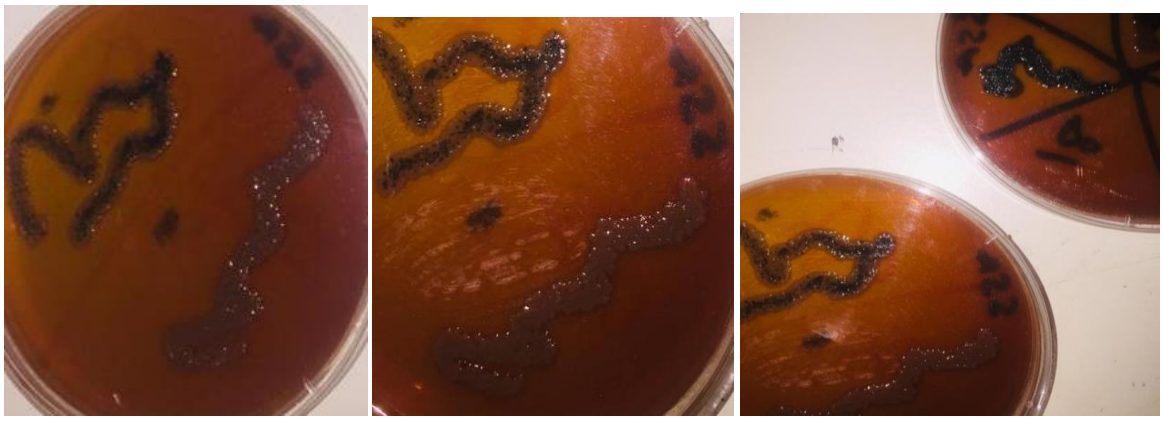
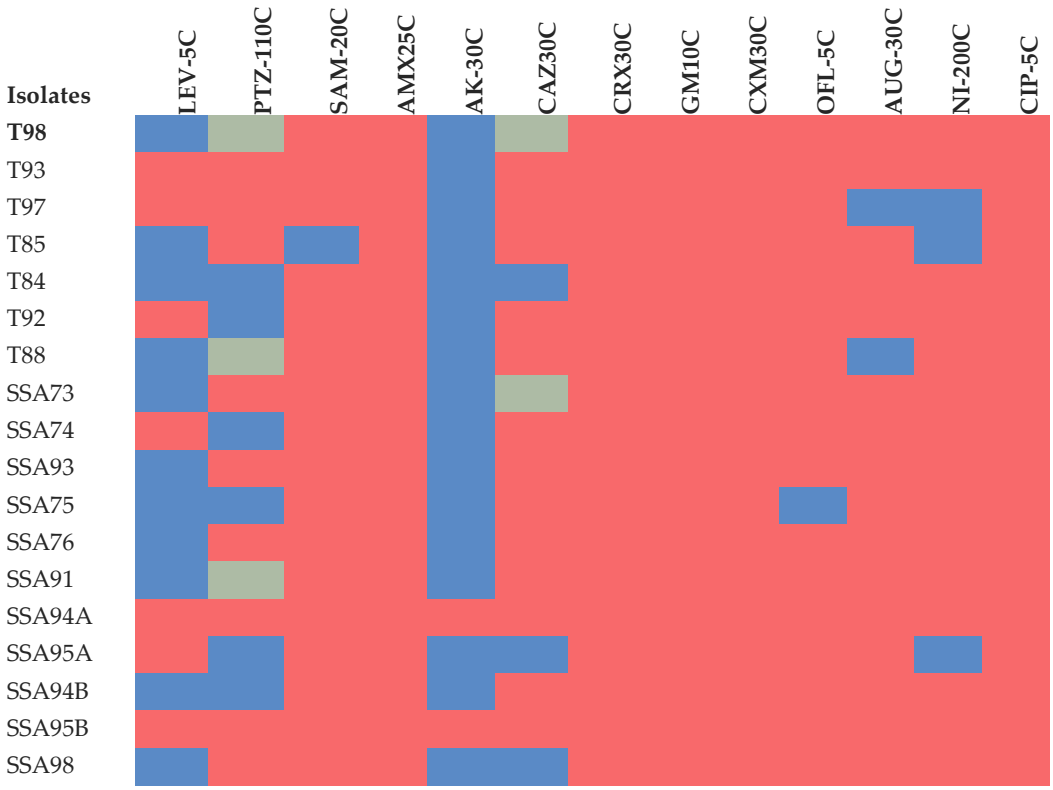


Figure 5: Cultural characters on Salmonella-Shigella Agar were hydrogen sulphide production of Salmonella species was observed during study

Figure 6: Cultural characters on Centrimide-Pseudomonas Agar were red pigmentation and green-blue colouration of Pseudomonas and Bulkhoderia species was observed during study



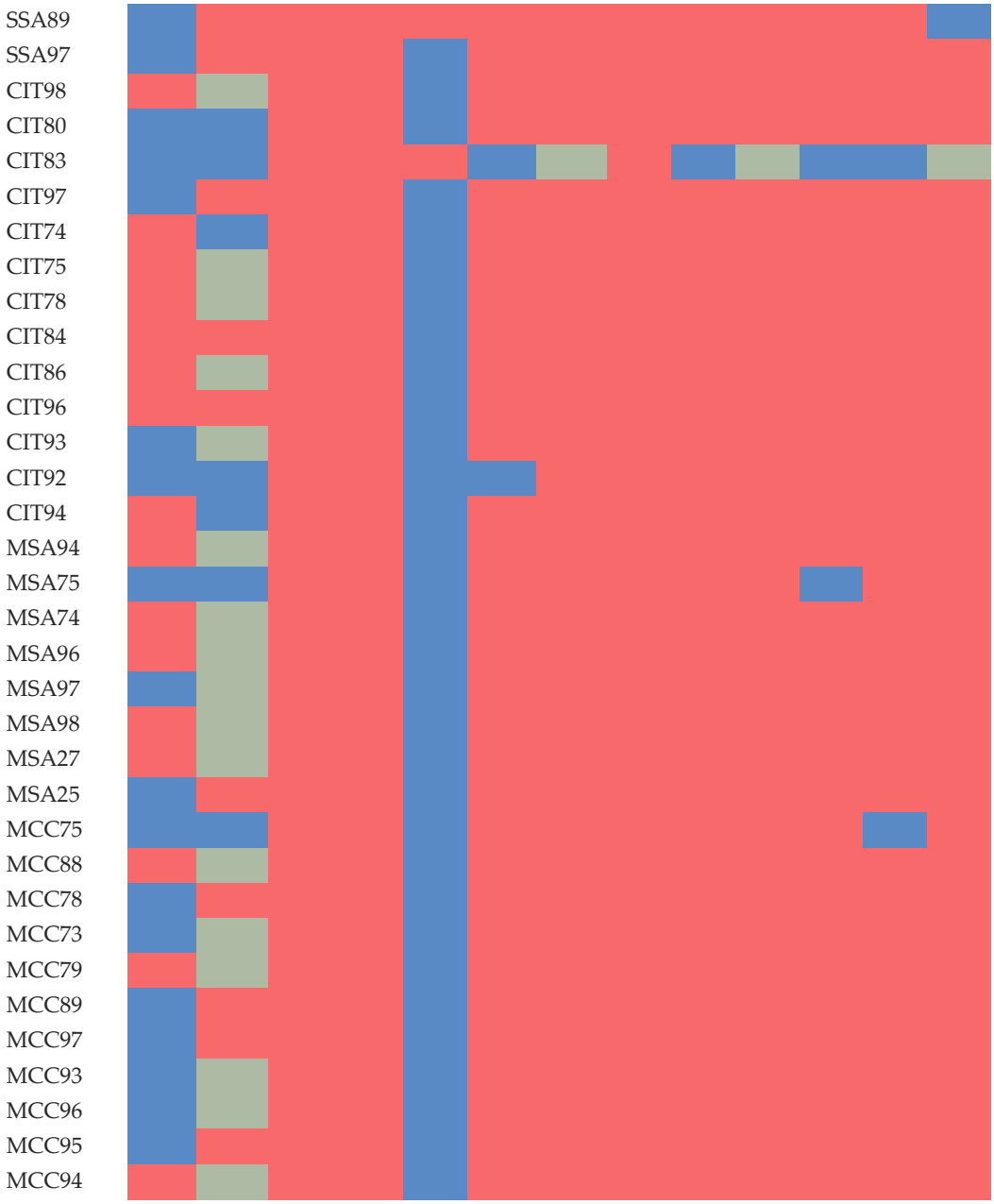


Figure 7: The antibiogram of the various presumptive ESKAPE-B isolates to relevant antibiotics. The various colour reads as follows: red indicates Resistance heat region, Gray is intermediate heat region while blue indicates sensitive heat region of map.

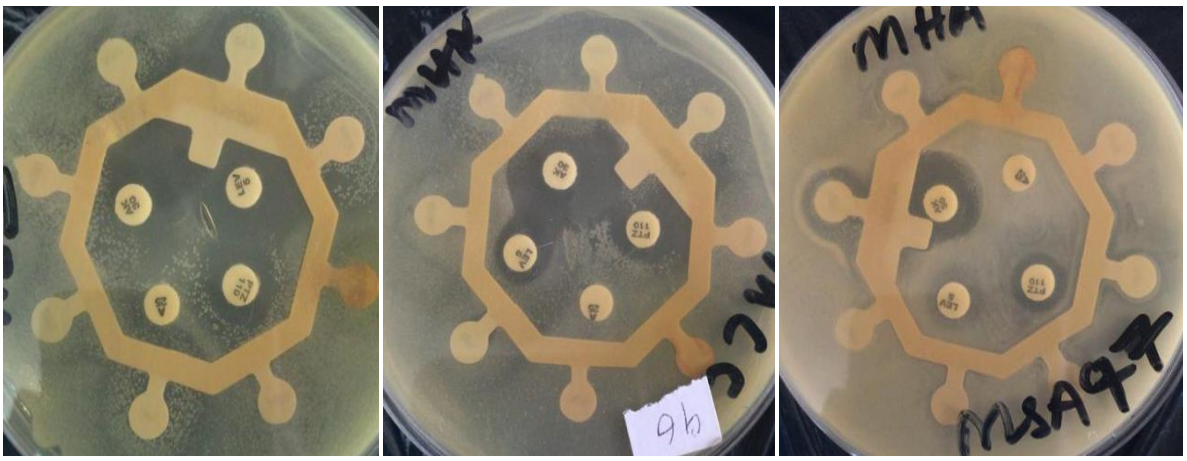


Figure 8a: Antibiotic Susceptibility test plates of Isolates observed during study

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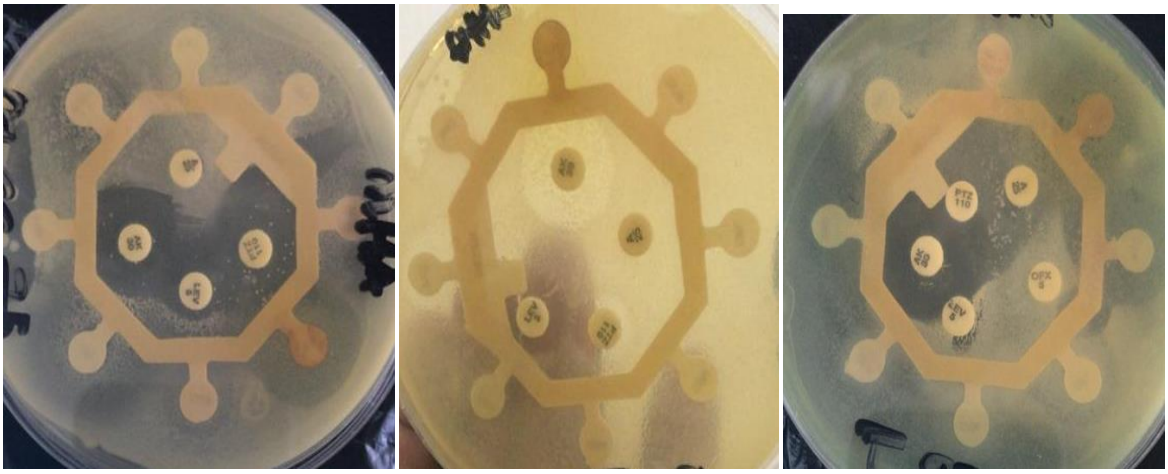

Figure 8b: Antibiotic Susceptibility test plates of Isolates observed during study

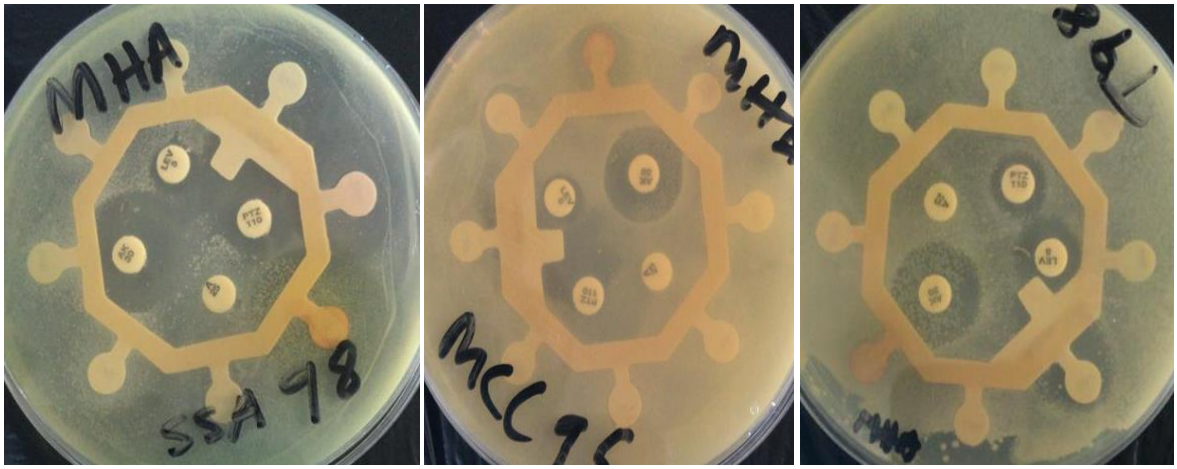

Figure 8c: Antibiotic Susceptibility test plates of Isolates observed during study

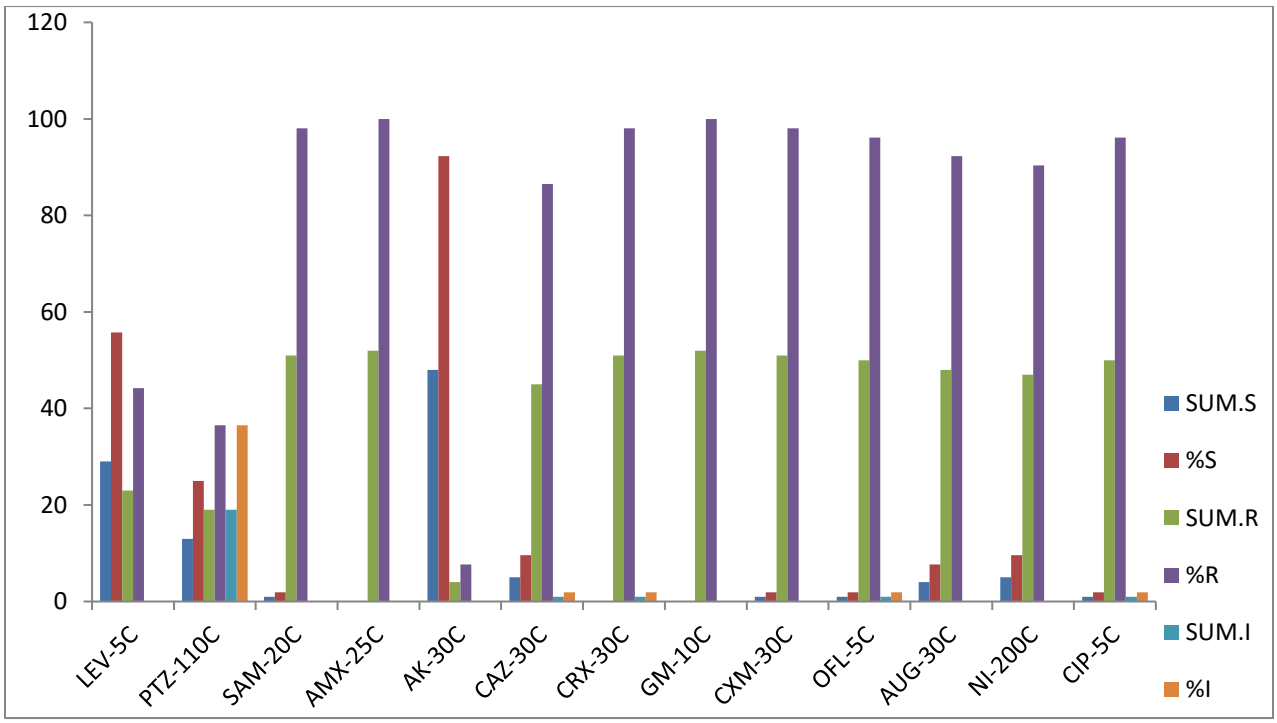

Figure 9: Isolates Percentage Sensitivity to the applied antibiotics observed during study

Table 4: Multiple antibiotic resistant index and Susceptibility Numbers

Isolates	NO. R	NO.I	S.NO	MARI
T98	9	2	2	0.692308
T93	12	0	1	0.923077
T97	10	0	3	0.769231
T85	9	0	4	0.692308
T84	9	0	4	0.692308
T92	11	0	2	0.846154
T88	9	1	3	0.692308
SSA73	10	1	2	0.769231
SSA74	11	0	2	0.846154
SSA93	11	0	2	0.846154
SSA75	9	0	4	0.692308
SSA76	11	0	2	0.846154
SSA91	10	1	2	0.769231
SSA94A	13	0	0	1
SSA95A	9	0	4	0.692308
SSA94B	10	0	3	0.769231
SSA95B	13	0	0	1
SSA98	10	0	3	0.769231
SSA89	11	0	2	0.846154
SSA97	11	0	2	0.846154
CIT98	11	1	1	0.846154
CIT80	10	0	3	0.769231
CIT83	4	3	6	0.307692
CIT97	11	0	2	0.846154
CIT74	11	0	2	0.846154
CIT75	11	1	1	0.846154
CIT78	11	1	1	0.846154
CIT84	12	0	1	0.923077
CIT86	11	1	1	0.846154
CIT96	12	0	1	0.923077
CIT93	10	1	2	0.769231
CIT92	9	0	4	0.692308
CIT94	11	0	2	0.846154
MSA94	11	1	1	0.846154
MSA75	9	0	4	0.692308
MSA74	11	1	1	0.846154
MSA96	11	1	1	0.846154
MSA97	10	1	2	0.769231
MSA98	11	1	1	0.846154
MSA27	11	1	1	0.846154
MSA25	11	0	2	0.846154
MCC75	9	0	4	0.692308
MCC88	11	1	1	0.846154
MCC78	11	0	2	0.846154
MCC73	10	1	2	0.769231
MCC79	11	1	1	0.846154
MCC89	11	0	2	0.846154

MCC97	11	0	2	0.846154
MCC93	10	1	2	0.769231
MCC96	10	1	2	0.769231
MCC95	11	0	2	0.846154
MCC94	11	1	1	0.846154

3.1. Plasmid Analysis

Plasmid Analysis revealed that amongst 132 isolates of ESKAPE-B stas as well as *Vibrio specie*, *Salmonella specie* and *Shigella specie* retrieved during the study, 62.9% (83/132) were shown to harbour plasmid of size ranging from 22kb to 1kb. This is an indication that some of the resistance observed from the antibiotic susceptibility testing was associated with resistant plasmids. There is need to further continue on the study to affirm the characteristics of the plasmid by curing and transformation of the extracted extra-chromosomal DNA (plasmid) detected during the study.

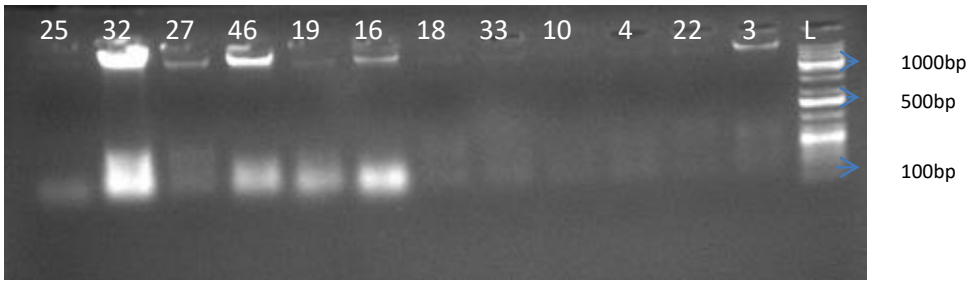

Figure 10a; Plasmid profile of multiple drug resistance ESKAPE-B, Vibrio specie, *Salmonella specie and Shigellas* analyzed with 0.8% agarose gel electrophoresis. L is 100bp-1kb ladder (molecular marker). Lanes 25, 32, 27, 46, 19 and 16 are positive for plasmid DNA at 100bp while Lanes 18, 33, 10, 4, 22 and 3 are negative for plasmid DNA.

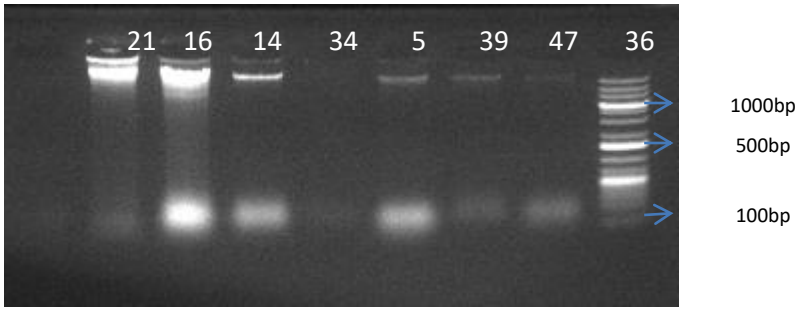

Figure 10b; Plasmid profile of multiple drug resistance *Salmonella species* analyzed with 0.8% agarose gel electrophoresis. L is 100bp-1kb ladder (molecular marker). Lanes 36, 47, 34, and 14 are positive for plasmid genes at 120bp, isolates 39 and 16 harbors plasmid genes at 100bp and Lanes 5 and 21 are negative for plasmid genes.

4. DISCUSSION

Reports on the spread of resistant bacteria strains in both hospital and the environment continues to heighten yet there is dearth of information on source tracking of such bacteria strains that thrives in the environment. Although the ubiquitous nature of bacteria and other microorganisms remains a natural phenomenon required for the survival of bacterial, it has become an area of concern for the public who negates the real life standard hygienic practice during domestic activities. In addition, multiple antibiotic resistant bacterial (MARB) or Superbugs are also on the increase. These groups (superbug) of organisms according to the unified acronym of microbiological studies are referred to as ESKAPE-B: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumanii, Pseudomonas aeruginosa, Enterobacter cloaca* and *Bulkhoderia cepacia* (CDC, 2009). Our study focuses on the role and influence of domestic activities in the spread of superbugs (multiple antibiotic resistant bacteria). The study which focuses on two assessment cluster namely: the determination of human subject awareness on the spread of multiple antibiotic resistant bacteria while the other cluster focuses on laboratory isolation, characterization, antibiogram and prevalence of the various organisms in selected domestic activities sources of human.

4.1. Awareness

Majority of persons recruited in the study area were mainly students (70) with age range 26-30 (36), males (60). Other persons are farmers, drivers and few educated persons as revealed from the questionnaire in Table 1, 2 and Fig 1. It was observed that most of them do not apply appropriateness in hygienic practice which is a potential source to resistant bacteria blume as well as spread within the area. The domestic activities of these persons were ranked as education, traveling, visitation while attending school {education (60/102)} and travelling (32/102) ranked the most with 58.8% and 31.4% of them involved as shown in Table 2, whereas their major water source was shown to be others (52/102; 51.0%) and Tap running water (40/102; 39.2%) while some of them applies no specific treatment of water. The toilet system mostly used was W/C standard (49/102; 48.03%), pithole toilet (40/102; 39.2%), while random disposal is 12.7% (13/102). Hygiene and other domestic activities revealed that most of them do not apply appropriate hygienic practice both at home and within their immediate environment. This is an indication that persons in the study area have poor knowledge of hygiene and relevance of hygienic practice. The study of numerous investigators have also previously reported that poor adherence to hygienic practice, poor disposal of waste, as well as inappropriate usage of antibiotics in treatment of bacteria associated infections are possible factors that encourage the spread/thrive of multiple antibiotic resistant bacteria in any environment (Arzanlou *et al*., 2017; CDC, 2015; Baquero *et al*., 2013, 2015; Allen, 2014; Chang *et al*. 2010; Magill *et al*., 2009; Sotgui *et al*., 2009; Udo *et al*., 1993). In addition, it was also reported in 2006 by WHO and UNICEF that the Sub-Saharan Africa (with 31%) is one notable region which is characteristic with poor coverage of improved sanitation and/or hygienic practice, followed by Southern Asia (33%) and Eastern Asia (65%) which have the highest poor hygiene application (WHO/UNICEF, 2013; CDC, 2009).

4.2. Presumptive Isolation, characterization and prevalence

Of the 132 isolates retrieved from culture onto selective, differential, and basal media for the members of the superbugs, the following were observed: *Enterococcus specie* (17/132; 12.9%), *Staphylococcus aureus* (20/132; 15.2%), *Klebsiella pneumonia* (10/132; 7.6%), *Acinetobacter baumanii* (11/132; 8.3%), *Pseudomonas aeruginosa* (21/132; 15.9%), *Enterobacter cloaca* (12/132; 9.1%) and *Bulkhoderia cepacia* (14/132; 10.6%). Other bacteria strains observed are *Vibrio cholerae* (12/132; 9.1%), *Salmonella specie* (10/132; 7.6%) and *Shigella specie* (6/132; 4.6%) as shown in Table 3. The figures 2, 3, 4, 5, 6 and 7 shows the photo-microgram of isolates cultural characteristics and morphological features. From the above observation, it is evident that *P. aeruginosa* is most prevalent in the environment with other members of the ESKAPE-B strains. In addition, other emerging bacterial strains were observed which is of relevance to public health and environmental wellness. Although the members of the ESKAPE-B strains are rarely reported as a group from environmental sources, they have been reported individually by various investigators as environmental/domestic contaminants (Igere et al., 2020; Manaia *et al*., 2018; Arzanlou *et al*., 2017; Baquero *et al*., 2013, 2015; Allen, 2014; Ashbolt *et al*., 2013). The study of Lederberg, Nordmann, Guo and other research colleagues revealed that *Enterococcus faecium* is prevalent in the environment (Lederberg, 2013; Nordmann *et al*., 2018; Guo *et al*., 2011; Edey *et al*., 2010; Toru *et al*., 2018; Rodriguez, 2016; Low *et al*., 2001). Other studies also reported the presence of *Staphylococcus aureus, Vibrio cholerae, Klebsiella pneumonia,Salmonella specie, Acinetobacter baumanii,Shigella specie, Pseudomonas aeruginosa, Enterobacter cloaca* and *Bulkhoderia cepacia* as potential environmental contaminants (Igere *et al*., 2020; Manaia *et al*., 2018; Arzanlou *et al*., 2017; Moon *et al*., 2007; Nam *et al*., 2011; Chishimba *et al*., 2016; Ismail and Haydar, 2016; Munoz-Price *et al*., 2008; Perez *et al*., 2007; Alnour *et al*., 2017; Delmar *et al*., 2014; Dayan *et al*., 2013; CDC, 2009; Shreve *et al*., 1999; Vandamme *et al*., 1997). It is important to note that some of these organisms were isolated from domestic toilet facilities, gymnastic homes, public transport vehicle seats, intra-city tricycle seats, classroom seats and office door handle. The door handle, seat of vehicles/classrooms/offices and gymnastic centre showed that the resistant bacteria thrives in these regions which utilizes the advantage of poor hygienic practice to survive and spread in these sources. This is similar to the report of some investigators on application of hygiene in the environment (Assembly, 2019; United Nation, 2019). It was reported by the united nations that diverse populations (844 million people) lacks proper sanitation facilities and do not have access to quality and portable drinking water. This has resulted to an annual death of 700,000 children and many leaving with ill health conditions, poor physical health and cognitive development in developing countries (Assembly, 2019; United Nation, 2019; Adams *et al*., 2016; Fanucchi, 2016; DeNavas-Walt and Proctor, 2014).

4.3. Phenotypic Virulent indices of bacterial strains

Although the bacteria strains isolated were environmental strains, a culture dependent virulence of the isolates was determined. The study accessed enzyme-based virulent determinants such as oxidase, protease, lipase, catalase, coagulase, and hemolysin as revealed during the study. A high member of isolates were shown to produce protease (94/132; 71.2) while other are catalase (91/132; 68.9%), oxidase (53/132; 40.2%), lipase (67/132; 50.8%), coagulase (25/132; 18.9%), and hemolysin (44/132; 33.3%). Similar phenotypic virulent characters were also reported amongst the related isolates by various investigators (Moghaddam *et al*., 2014;

Ekundayo *et al*., 2019, Igere *et al*., 2020). Such virulent determinants are relevant patho-significant indices of the bacteria strains which are necessary for pathogenesis in any disease case where they may be found. Observing such virulent characters amongst environmental strains is an indication that these strains are potential pathogens, which calls for public health concern.

4.4. Antibiotic Susceptibility Profile

Following the CLSI interpretative guidelines for various *in vitro* antibiotics used, the bacterial strains were grouped into R (resistant strains), I (intermediate strains), S (sensitive strains) (CLSI, 2015). The Table 4 and figures 8, 9 and 10 shows the antibiotic profile and the interpretation of thirteen antibiotics applied during the study. The various colour reads on the figure 7 heat-map shows that red represents resistant region, grey represents intermediate region while blue represents sensitive region. The very broad region covered by the red map shows that majority of the isolates were multiple antibiotics resistant. The resistant phenotypes observed reveals as follows Ampiclox (AMX-25C; 100%), Ceftazidime (CAZ-30C; 86.5%), Cefuroxime (CRX-30C; 98.1%), Gentamycin (GM-10C; 100%), Cefixime (CXM-30C; 98.1%), Augmentin (AUG-30C; 92.3%), Nitrofurantoin (NI-200C; 90.4%), Ofloxacin (OFL-5C; 96.2%), Sulbactam-Ampicillin (SAM-20C; 98.1%) etc as revealed in figure 9. The figures 8 a, b, c shows the antibiotics disc invitro-test on the isolates which were interpreted based on the CLSI/EUCAST interpretative standard. It is important to note that these reported antibiotics are routine antibiotics commercially applied in the treatment and management of infections implicated by the observed organisms. Observing such high level resistant phenotype is an indication of spread and sharing of these resistant genes within the environment. The isolates were shown to be multiple antibiotics resistant to the various groups of antibiotics such as β-lactam, cephalosporin, β-lactam inhibitors, fluoroquinolone, aminoglycoside, macrolide and nitrofuran. The observation of these high resistant bacterial members affirms that the isolates are superbugs and are distributed within various environmental and domestic sources. This is a call for public health interest as it is suggestive that attention and interest towards limiting or eradicating such potential clinically relevant pathogens be instituted. The study of various investigators on ESKAPE-B and other pathogens has also reported similar multiple antibiotic resistant phenotype and genotypes amongst these group of bacteria (Igere *et al*., 2020; Velayati *et al*., 2009; Magiorakos *et al*., 2012; Sievert *et al*. 2013; Ashbolt *et al*., 2013). The high resistant strains observed during the study may be associated with the poor awareness of the thriving tendency of resistant strains (Superbugs) in the environment and domesticated sources. It may also be associated with inappropriateness in the application of standard hygienic practice by the subjects in the study area which inform the need for appropriate application of hygiene. The Table 4 also shows the numbers of resistant and susceptible isolate to the applied antibiotics with a high multiple antibiotic resistant index (MARI) ranging from 0.6 to 1. Such high MARI indicates that isolates has acquired diverse members of multiple antibiotics resistant phenotype affirming the isolates as Superbugs. The multiple antibiotic resistant natures of observed isolates were further affirmed by their plasmid profile. Plasmid Analysis revealed that amongst 132 isolates of ESKAPE-B bacteria as well as *Vibrio specie*, *Salmonella specie* and *Shigella specie* retrieved during the study 62.9% (83/132) were shown to harbour plasmid of size ranging from 22kb to 1kb. This is an indication that some of the resistance observed from the antibiotic susceptibility testing was associated with resistant plasmids. There is need to further continue on the study to affirm the characteristics of the plasmid by curing and transformation of the isolated extra-chromosomal DNA (plasmid) detected during the study.

5. CONCLUSION

The Surveillance and assessment of potentially pathogenic bacteria found in shared public resources and domesticated sources is an important component of research which is aimed at understanding, preparing for, and protecting communities from potential widespread infection and transmission of infectious diseases. It is also an important determinant for the control of fast spreading resistant strains (Superbugs) in various environments. The distribution and spread of such bacteria has been traceable to the poor implementation of standard hygienic practice and poor awareness on the possibility that non-adherence to hygienic practice may encourage survival and thriving of resistant bacteria strains. It does not also negate the need for appropriate usage of antimicrobial agent as well as disposal of waste. The assessment of environmental and domestic activities for spread of multiple antibiotic resistant bacteria using the applied methods during this study has emerged as an efficient, cost-effective, and reliable method for assessing and estimating diverse population on the level of multiple antibiotic resistant related organisms.

Recommendation

It is suggestive that a planned repeated surveillance of the environment for emerging resistant strains be continuous in diverse environment as there is a possibility of a replicated report in other nearby area. The observations of such resistant strains also inform the need for appropriate application of interpersonal hygiene. The study has revealed the assessment of multiple antibiotic

resistant bacteria in the environment since environmental and domestic activities has been implicated as influencing dynamics in the environment. It is recommended that a routine and periodic awareness campaign on appropriate application of inter-personal hygiene must be instituted

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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